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LETTERS AND CORRESPONDENCE

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Transitional Pre-B-Cell Acute Lymphoblastic Leukemia in Adults

To the Editor: Blast cells of <2% of cases of pediatric B-progenitor cell acute lymphoblastic leukemia (B-ALL) display transitional pre-B (TPB) cell immunophenotype defined by the presence of cytoplasmic (c) and surface (s) μ heavy chains but not light (κ/λ) chains [1,2]. Children with transitional pre-B-cell acute lymphoblastic leukemia (TPB-ALL) have a very favorable prognosis, lack French-American-British (FAB) L3 morphology and bulky extramedullary disease [1]. To the best of our knowledge, data concerning the demographic, clinical, and laboratory features of TPB-ALL in adults have not been reported. Thus, this letter describes the presenting characteristics of four adults with TPB-ALL identified in a cohort of 60 untreated patients with de novo acute leukemia consecutively diagnosed in a 20-month period (March 1997 to October 1998).

In all patients, immunologic cell typing was performed in whole bone marrow collected in ethylenediaminetetraacetic acid. Aliquots containing ~10^6 nucleated cells were incubated with a panel of monoclonal antibodies directed to: leukocyte common antigen (CD45), B-lymphoid-associated antigens (CD19, CD20, and CD22), common acute lymphocytic leukemia antigen (CD10), κ and λ light chains, T-lymphoid-associated antigens (CD2, cCD3, CD5, and CD7), myeloperoxidase (cMPO), myeloid-associated markers (MAM; CD13, CD14, CD15, CD33, and CD41), pluripotential stem-cell antigen (CD34), terminal deoxynucleotidyl transferase (TdT), and HLA-DR monomorphic antigen (DR). $F(ab')_2$ rabbit anti-human IgG and IgM antibodies were used to identify γ and μ heavy chains. TdT and CD22 were assayed for by indirect immunofluorescence and all other cell markers were evaluated by direct immunofluorescence technique. Unfixed stained cells were immediately analyzed for fluorescent activity by flow cytometry (Cytoron Absolute, Ortho Diagnostic Systems, Raritan, NJ). Positivity of CD19, CD20, and/or CD22 defined B-ALL. Cytogenetic analysis was performed in three patients and was unsuccessful in all 3. Patients with early pre-B-, pre-B-, and TPB-ALL were treated with the same chemotherapy schedule.

TABLE I. Demographic, Clinical, and Laboratory Features of Adults with Transitional Pre-B ALL

	Patient 1	Patient 2	Patient 3	Patient 4
Sex/Age (yr)	female/33	male/23	female/57	female/41
Bleeding	no	retina, GI	no	uterus, MC
LNE[a]	~1.5 cm	no	~1.0 cm	no
Splenomegaly	no	2 cm	no	5 cm
Hepatomegaly	no	no	no	6-8-10 cm
WBC (10^9/l)	50.0	4.2	23.2	3.8
FAB/Blasts (%)	L2/70	L2/98	L2/99	L1/98
$CD19^+$ (%)	**67**[b]	**57**	**76**	**73**
$CD20^+$ (%)	13	14	**42**	12
$CD22^+$ (%)	**37**	**85**	**86**	**68**
$c\mu^+/s\mu^+$ (%)	**44/34**	**94/39**	**36/21**	**92/66**
$s\gamma^+$ (%)	5	1	15	19
κ^+/γ^+ (%)	0.6/0.8	1.5/0.6	4.2/1	2.5/0.7
$CD10^+$ (%)	**95**	**89**	**92**	**44**
$CD34^+$ (%)	**96**	**92**	**83**	1
TdT^+ (%)	**71**	**96**	**76**	**71**
DR^+ (%)	**98**	**96**	**89**	**85**
FU (days)	589	41	11	35
Outcome	alive, CR, CT	alive, IT	dead	alive, IT

[a]Abbreviations: [a]GI = gastrointestinal, MC = mucocutaneous, LNE = lymph node enlargement, WBC = white blood cell count, FAB = French-American-British morphologic classification, FU = follow-up, CR = complete remission, CT = continuation therapy, IT = induction therapy.
[b]Numbers in bold represent positive results.

Among 27 cases of B-ALL, 14 had early pre-B immunophenotype ($c\mu^-$, $s\mu^-/s\gamma^-$), 9 were pre-B ($c\mu+$, $s\mu^-/s\gamma^-$, κ^-/λ^-), and 4 cases (14.8%) had phenotypic properties of TPB-ALL (Table 1). Clinical neurologic abnormalities and blasts in cerebrospinal fluid were absent in all patients. Case 4 presented spontaneous tumor lysis syndrome. As reported in children, transitional lymphoblasts in all our patients lacked FAB L3 morphology [1,3], expressed TdT nuclear enzyme [1,3], HLA-DR, CD10 [1,3,4], as well as, CD19 and CD22 antigens [1]. Interestingly, leukemic cells from case 1 expressed MAM (CD33 = 70% and cMPO = 29%). Furthermore, double-color cytometric analysis showed that 91% and 58% of her blasts were $CD10^+/CD33^+$ and $CD19^+/CD33^+$, respectively.

At the present time it is premature to draw any conclusion concerning treatment outcome in our patients with TPB-ALL (Table 1). More reports on similar cases are needed to improve our understanding of the prognosis for this patient's subset and ameliorate our knowledge of the heterogeneity of B-ALL. In summary, our data show that: i) lymphoblasts displaying TPB immunophenotype are present in adult leukemic patients; ii) apparently the frequency of TPB-ALL in adults is higher than in childhood B-ALL; and iii) mixed-lineage acute leukemias [5] also comprise TPB-ALL.

Xavier López-Karpovitch
Josefa Piedras
Hematology-Oncology Department, Laboratory of Cell Biology, Instituto Nacional de la Nutrición Salvador Zubirán, Mexico, D.F.

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Mixed Connective Tissue Disease with Hemolytic Anemia and Severe Thrombocytopenia due to Thrombotic Thrombocytopenic Purpura

To the Editor: In 1972, Sharp et al. [1] described an apparently distinct overlap syndrome characterized by clinically features of 2 or more connective tissue diseases in association with a high titer of antibodies to a ribonuclear protein (anti-RNP), which they called mixed connective tissue disease (MCTD). Because of its rarity we report the case of a patient who developed thrombotic thrombocytopenic purpura (TTP) associated with MCTD.

In July 1989, a 40-year-old black woman was admitted to the hospital because of fever, Raynaud phenomenon, swollen fingers, polyarthritis, and cutaneous rash. Laboratory examination disclosed positive antinuclear (1:10,000 with a speckled pattern), and anti-RNP (1:640) antibodies. DNA binding by Farr assay was negative. Skin biopsy disclosed lymphocytic vasculitis, nailfold capillary microscopy showed increased number of enlarged loops, extravasates and areas with lacking capillaries. Hydroxychloroquine therapy at 400 mg/day was started, and over the ensuing months, non steroïdal anti-inflammatory drugs, were added to control the manifestations of MCTD. In June 1991, she presented with headache, confusion, seizures, easing bruising, and fever. The physical examination showed a blood pressure of 150/70 mm Hg, diffuse petechiae and ecchymoses, the temperature was 40.7°C. No focal neurologic sign was detected, and cerebral computerized scan was normal. Laboratory studies showed the following results: erythrocyte sedimentation rate 80 mm/h, white blood cell count 9.38×10^9/l, platelet count 9×10^9/l, hemoglobin 5.4 g/dl, schistocytes 15%, reticulocytes count 10%, lactate dehydrogenase activity 3560 UI/l (normal < 320), serum haptoglobin 0.05 g/l (normal, 0.6–1.8 g/l). Renal function and coagulation tests were normal. Antibodies to human immunodeficiency virus and a direct Coombs' test were negative. A diagnosis of TTP was made, and detailed study failed to identify any cause of thrombotic microangiopathy other than MCTD. The patient was given 2 mg of vincristine sulfate intravenously and was then treated daily by 4 l plasma exchanges, dipyridamole 600 mg/day, aspirin 250 mg/day, and prenisolone 100 mg per day. Biological abnormalities improved after three plasma exchanges. The patient became acutely ill on the 10th hospital day with relapse of thrombocytopenia ($10{,}000/mm^3$), hypoxia and refractory hypotension. She died on the 12th hospital day. Permission for autopsy was denied.

The association between TTP and MCTD has been previously reported in only 2 patients [2,3]. Nevertheless, it is important to recognize TTP as a cause of thrombocytopenia and hemolytic anemia because treatment differs from steroid therapy usually used in case of autoimmune cytopenia due to active MCTD. In this observation, vincristine, prednisolone, antiplatelet agents, and plasma exchange resulted in temporary improvement. However, TTP recurred and the patient died of uncontrolled manifestations of thrombotic microangiopathy. Since depressed plasma fibrinolytic activity and cytotoxic endothelial cell antibodies, two mechanisms accounting for thrombotic microangiopathy, have been reported in patients with MCTD [4,5], and since we failed to identify any other underlying factor, we postulate that vasculitis related to MCTD may have been involved in the pathogenesis of TTP in our patient. The association between TTP and connective tissue disease provides additional evidence that endothelial cells are involved in the disease process. Although of rare occurence, it is important to recognize TTP as a cause of thrombocytopenia and hemolytic anemia in MCTD, because a specific therapy is required.

Pascale Poullin
Patrice Lefevre
Jean Marc Durand
Department of Hemobiology, Hopital de la Conception, Marseille, France
Department of Internal Medicine, Hopital Sainte-Marguerite, Marseille, France

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Absence of C282Y and H63D Mutations of the Hemochromatosis Gene in Japanese Patients with Sideroblastic Anemia

To the Editor: It has been reported that 87% patients with hemochromatosis (HC) were homozygous for C282Y or compound heterozygous for C282Y/H63D mutations of the HFE gene [1]. More recently, C282Y and H63D mutations of the HFE gene have also been reported in other iron-overload diseases such as porphyria cutanea tarda (PCT), and sideroblastic anemia (SA) [2]. SA is characterized by microcytic hypochromic anemia with numerous ringed sideroblasts in bone marrow, and occurs both as an inherited and an acquired form. A hereditary form, X-linked sideroblastic anemia (XLSA), has been shown to be due to mutations within the erythroid specific δ-aminolevulinate synthase (ALAS-E) gene, while the cause of acquired SA is poorly understood except for drug-induced SA [3]. The major cause of death in hereditary SA patients is complications due to iron overload. While the prevalence of HC in European derived population is about 3 per 1,000, it is considerably less in other population [4]. With this view in mind, we examined the incidence of the C282Y and H63D mutations of the HFE gene in Japanese patients with SA.

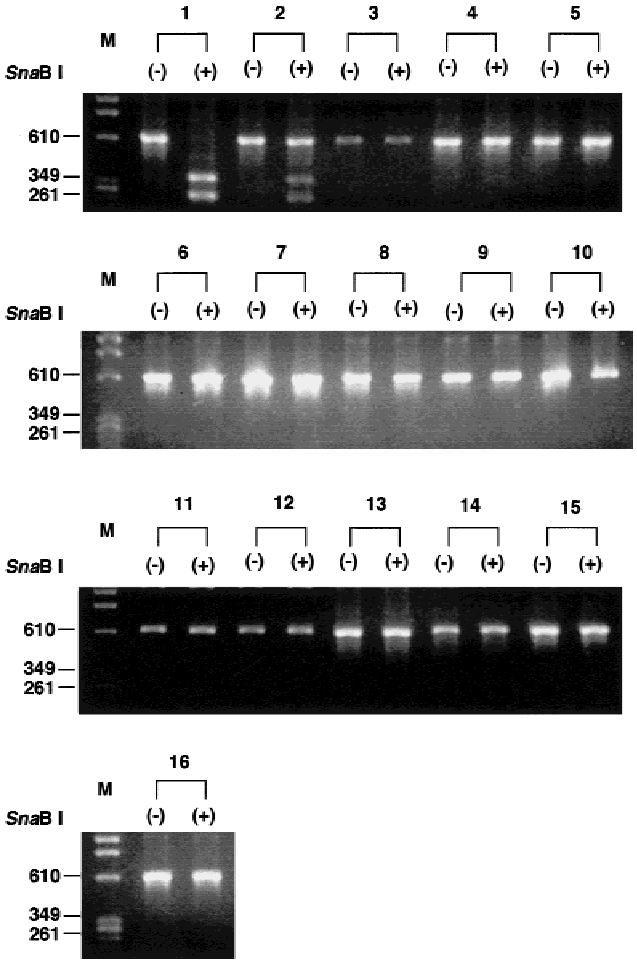

Fig. 1. The 4th plus the 5th exon with the 4th intron of the HFE gene, which had been amplified by PCR, was purified by agarose gel electrophoresis, and then incubated with (+), or without (−) *Sna*BI. Because the C282Y mutation creates a new *Sna*BI restriction site, the PCR product (610 bp) of the mutant DNA yields two fragments with the size of 349 bp and 261 bp. Sample M, the size marker; 1, C282Y homozygote; 2, C282Y heterozygote; 3 and 16, normal; 4–6, XLSA; 7–11, hereditary SA; 12–15, acquired SA. Samples 1 and 2 were C282Y positive controls.

We examined a total of 14 subjects (12 males and 2 females, age ranging from 4 months to 75 years old), including two patients with XLSA, a patient with suspected XLSA with an ALAS-E mutation, five patients with congenital SA without ALAS-E mutations, four patients with refractory anemia with ringed sideroblasts, and two normal volunteers. Genomic DNA was extracted from blood samples, with an informed consent, using a standard technique as described previously [5]. The 4th plus the 5th exon with the 4th intron, and the 2nd plus the 3rd exon with the 2nd intron of the HFE gene, were amplified independently. Because C282Y mutation creates a new *Sna*BI restriction site in exon 4, while H63D mutation disrupts the *Bcl*I restriction site in exon 2, these restriction sites were determined by examining restriction fragment length polymorphism using *Sna*BI and *Bcl*I. The results of our study clearly show that neither C282Y (Fig. 1), nor H63D mutation (data not shown) was detected in any of the 14 subjects we examined.

C282Y and H63D mutations of the HFE gene have been identified in many patients with HC and PCT. In particular, C282Y homozygosity was found at 80–90% in HC patients and at approximately 20% in PCT patients of European origin. Most recently, the C282Y mutation was also implicated in patients with XLSA [2]. Our study in Japanese patients, however, did not show the HFE mutations in a total of 12 patients with SA, even in seven patients who were severely iron-overloaded. This finding may most likely reflect a low incidence of the HFE gene in the Japanese population, but also indicates the fact that many patients with SA develop ringed sideroblasts independent of association with the HFE gene mutations, and that there may be difference in geographic genetic contribution to the development of iron-overload diseases such as SA.

K. Furuyama
M. Kondo
H. Fujita
N. Hayashi
K.E. Anderson
S. Sassa

The Rockefeller University, New York, New York
Tohoku University School of Medicine, Sendai, Japan
The Institute of Public Health, Tokyo, Japan
Hokkaido University School of Medicine, Sapporo, Japan
University of Texas Medical Branch, Galveston, Texas

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